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Pluteus losulus, a new record from south China

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Abstract—*Pluteus losulus* is reported as a new record from China. It is characterized by its low umbo with brown to grayish-brown pileus, hooked and metuloid pleurocystidia, variable cheilocystidia, presence of caulocystidia, and frequent presence of clamp connections in the pileipellis. New data from the nuclear ribosomal internal transcribed spacer (ITS) region place this species in a sub-basal clade of *P.* sect. *Pluteus*. A morphological description, illustrations, and phylogenetic placement of the Chinese *P. losulus* collection are provided.

Key words—basal clade, *Pluteaceae*, taxonomy

Introduction

Pluteus Fr. sect. *Pluteus* accommodates species with mainly metuloidal hymenial cystidia and a cutis type pileipellis, including the generic type *P. cervinus* (Schaeff.) P. Kumm. [= *Agaricus pluteus* Batsch: Fr.] (Singer 1986; Justo & al. 2011a,b, 2014). In past decades, some taxonomic studies with *Pluteus* distributional records have been reported from China (Teng 1963; 1996; Tai 1979; Bi & al. 1993; Yang & al. 2010, 2011; Xu & al. 2015). However, the fungal resources of this genus have not yet been comprehensively studied for this country.

In an effort to document the *Pluteus* species in China, several collections of *Pluteus* were made in 2017, one of which represented a member of *P.* sect. *Pluteus*. Based on morphological observation and molecular evidence, the examined specimen was identified as *P. losulus*. This species was originally described as *P. cervinus* var. *ealaensis* from Democratic Republic of Congo, Africa, and was elevated to species rank by Justo & al. (2011a) under the replacement name *P. losulus*. Phylogenetically, *P. losulus* was assessed as a basal taxon of *P.* sect. *Pluteus* and sister to *P. atromarginatus* (Justo & al. 2011a). In this study, we describe *P. losulus* as a new record for China. This species is compared here with the basal/sub-basal taxa of *P.* sect. *Pluteus*, and its phylogenetic position is further assessed based on the nuclear ribosomal internal transcribed spacer (ITS) region.

Material & methods

Morphological studies

The specimen was collected from south China (Dinghushan Biosphere Reserve Forest, Zhaoqing City, Guangdong Province, China), described, and deposited in the Fungal Herbarium, Guangdong Institute of Microbiology, Guangzhou, China (GDGM). Macro- and microscopic terminology followed Vellinga (1988).

Macromorphological descriptions were based on the field notes and documented with photographs. Micromorphological observations were made from the dried specimens mounted in H_2O, 5% aqueous KOH (w/v), and Congo Red. Melzer's solution was used to check any amyloid reaction of basidiospores and tissues. Line drawings were freehand. In the descriptions of basidiospore measurements, the notation [n/m/p] was used, which means 'n' basidiospores from 'm' basidiomata of 'p' collections. Dimensions for basidiospores are given as (a–)b–c(–d), in which 'b–c' contains a minimum of 90% of the measured values and extreme values 'a' and 'd' are given in parentheses, when applicable. Q denotes the length/width ratio of a measured basidiospore, and Q_m denotes the average of 'n' basidiospores (presented as mean ± standard deviation).

Molecular studies

Protocols for genomic DNA extraction, PCR amplification, and sequencing followed Hosen & al. (2013) and references cited there. The ITS1-F/ITS4 primer pair (White & al. 1990) was used for the amplification of ITS region.

Representative ITS sequences of *Pluteaceae* (46 samples of *Pluteus* and one sample of *Volvopluteus* Vizzini & al. as outgroup) were retrieved from GenBank for phylogenetic analysis. Sequences were included in phylogenetic analysis based on NCBI blast search results and published molecular analyses (Menolli & al. 2010, 2015a,b; Justo & al. 2011a,b, 2014; Pradeep & al. 2012; Malysheva & al. 2016; Desjardin & Perry 2018), and then combined with the newly generated sequence of the Chinese *Pluteus* collection (GDGM47122). The ITS sequences were aligned in

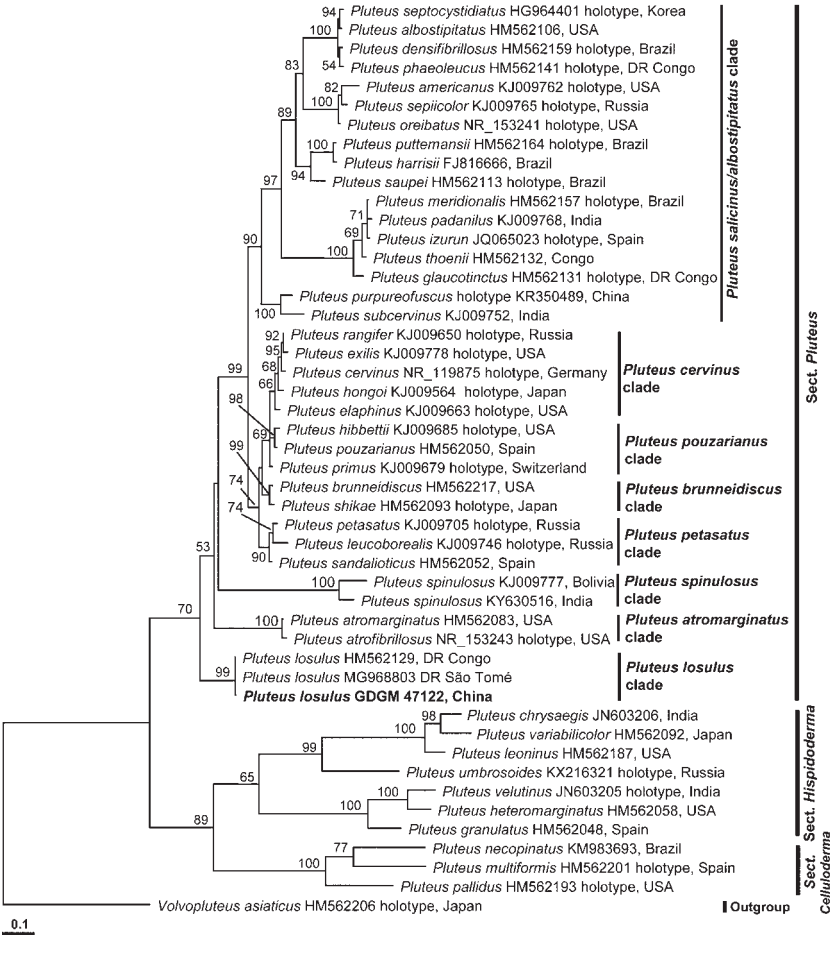
Pluteus septocystidiatus HG964401 holotype, Korea
Pluteus albostipitatus HM562106, USA
Pluteus densifibrillosus HM562159 holotype, Brazil
Pluteus phaeoleucus HM562141 holotype, DR Congo
Pluteus americanus KJ009762 holotype, USA
Pluteus sepiicolor KJ009765 holotype, Russia
Pluteus oreibatus NR_153241 holotype, USA
Pluteus puttemansii HM562164 holotype, Brazil
Pluteus harrisii FJ816666, Brazil
Pluteus saupei HM562113 holotype, Brazil
Pluteus meridionalis HM562157 holotype, Brazil
Pluteus padanilus KJ009768, India
Pluteus izurun JQ065023 holotype, Spain
Pluteus thoenii HM562132, Congo
Pluteus glaucotinctus HM562131 holotype, DR Congo
Pluteus purpureofuscus holotype KR350489, China
Pluteus subcervinus KJ009752, India
Pluteus salicinus/albostipitatus clade
Pluteus rangifer KJ009650 holotype, Russia
Pluteus exilis KJ009778 holotype, USA
Pluteus cervinus NR_119875 holotype, Germany
Pluteus hongoi KJ009564 holotype, Japan
Pluteus elaphinus KJ009663 holotype, USA
Pluteus cervinus clade
Pluteus hibbettii KJ009685 holotype, USA
Pluteus pouzarianus HM562050, Spain
Pluteus primus KJ009679 holotype, Switzerland
Pluteus pouzarianus clade
Pluteus brunneidiscus HM562217, USA
Pluteus shikae HM562093 holotype, Japan
Pluteus brunneidiscus clade
Pluteus petasatus KJ009705 holotype, Russia
Pluteus leucoborealis KJ009746 holotype, Russia
Pluteus sandalioticus HM562052, Spain
Pluteus petasatus clade
Pluteus spinulosus KJ009777, Bolivia
Pluteus spinulosus KY630516, India
Pluteus spinulosus clade
Pluteus atromarginatus HM562083, USA
Pluteus atrofibrillosus NR_153243 holotype, USA
Pluteus atromarginatus clade
Pluteus losulus HM562129, DR Congo
Pluteus losulus MG968803 DR São Tomé
Pluteus losulus GDGM 47122, China
Pluteus losulus clade
Sect. Pluteus
Pluteus chrysaegis JN603206, India
Pluteus variabilicolor HM562092, Japan
Pluteus leoninus HM562187, USA
Pluteus umbrosoides KX216321 holotype, Russia
Pluteus velutinus JN603205 holotype, India
Pluteus heteromarginatus HM562058, USA
Pluteus granulatus HM562048, Spain
Sect. Hispidoderma
Pluteus necopinatus KM983693, Brazil
Pluteus multiformis HM562201 holotype, Spain
Pluteus pallidus HM562193 holotype, USA
Sect. Celluloderma
Volvopluteus asiaticus HM562206 holotype, Japan
Outgroup
0.1

FIG. 1. RAxML tree representing phylogenetic relationships of *Pluteus* species inferred from ITS data. Bootstrap support values ≥50% from Maximum likelihood analysis are indicated on the branches at nodes. *Pluteus losulus* from China is highlighted in bold on the tree. GenBank accession numbers are provided after the species name followed by country of origin. The tree is rooted by *Volvopluteus asiaticus* Justo & Minnis as outgroup.

MAFFT v.6.8 (Katoh & al. 2005), and manually edited in BioEdit v.7.0.9 (Hall 1999) using default settings. Maximum Likelihood (ML) method was used to analyze ITS dataset. ML was performed in RAxML v.7.2.6 (Stamatakis 2006). All parameters in the ML analysis were used as default setting and statistical support values were obtained using nonparametric bootstrapping (BS) with 1000 replicates.

Molecular phylogenetic results

The newly generated ITS sequence of the Chinese *Pluteus* collection was deposited in GenBank (MH231231). In the aligned ITS dataset, sequences of the 48 samples were included with 757 nucleotide sites (gaps included) for each sample. The Chinese collection of *Pluteus* clustered in *P.* sect. *Pluteus* and grouped with African collections of *P. losulus* from Democratic Republic of Congo (GenBank HM562129) and São Tomé, strongly supported by ML analysis (Fig. 1). However, its phylogenetic relationship with other taxa/clade of this section is not satisfactorily resolved. The summarized result of the phylogenetic analysis is presented in Fig. 1.

Taxonomy

Pluteus losulus Justo, Mycol. Progr. 10: 473 (2011). Figs 2, 3

≡ *Pluteus cervinus* var. *ealaensis* Beeli, Bull. Soc. Roy. Bot. Belgique 61(1): 81 (1928).

Basidiomata small to medium sized. Pileus up to 45 mm broad, plano-convex to hemispherical with age, with a low umbo; surface uneven, slightly striate radially, covered with radially arranged fibrils, brown, dull brown, reddish brown or grayish-brown, slightly paler with age; margin entire, slightly sulcate, paler than center. Lamellae 4–5 mm broad, crowded, free, ventricose, pale white when young, becoming pink; lamellulae common, 2–3 tiers, truncate. Stipe 35 × 4 mm, cylindrical to narrowly obclavate, attenuated towards apex, surface covered with whitish to light grayish-brown fibrils; basal mycelium whitish. Context white, unchanged when cut or bruised. Odor and taste not recorded.

Basidiospores [25/1/1] (6.0–)6.5–7.5(–8.5) × 6.0–7.0 µm, [mean 7.11 × 6.40 µm; Q = 1.03–1.16(–1.21); Q_m = 1.11 ± 0.06] nearly globose to subglobose, rarely broadly ellipsoid, hyaline to pale yellowish in KOH and H_2O, smooth, thick-walled. Basidia 20–30 × 8–11 µm, 4-spored, clavate, smooth, thin-walled. Cheilocystidia 23–75 × 10–18 µm, abundant, crowded, variable, clavate to narrowly clavate, subcylindrical, fusoid, sometimes lanceolate to mucronate, hyaline, smooth, thin-walled. Pleurocystidia 60–100 × 15–25(–35) µm, abundant, metuloidal, fusiform to narrowly utriform, sometimes a deformed shape with bifid apices, occasionally with an outgrowth on lateral side, near lamellar edge hooks slightly developed or nearly rounded apices, hyaline. Pileipellis a cutis, terminal elements 75–125(–230) × 7–20 µm, cylindrical, narrowly fusoid to utriform, some strongly tapering towards apex, filled with brown intracellular pigments, smooth, thin-walled. Stipitipellis cutis, vertically oriented, hyphae 4–15 µm wide, cylindrical, pale

Fig. 2. *Pluteus losulus* (GDGM 47122). Basidiomata in natural habitat.

yellowish or colorless, thin-walled, smooth. Caulocystidia 40–80 × 12–20 (–25) µm, scattered, fusiform to broadly fusiform, lanceolate, utriform, clavate, with some brown intracellular pigments, thin-walled. Clamp connections present, common in the pileipellis.

Specimen examined: **CHINA**, **Guangdong province**: **Zhaoqing City**, Dinghushan Biosphere Reserve Forest, 221 m asl, 12 May 2017, leg. Md. Iqbal Hosen, Hao Huang & Xishen Liang (GDGM 47122, GenBank MH231231).

Habitat and distribution—Solitary, found on decayed wood of *Schima superba* Gardner & Champ. (*Theaceae*). Recorded from Democratic Republic of Congo (Justo & al. 2011a), Republic of São Tomé and Príncipe (Desjardin & Perry 2018), and China (this study).

Discussion

The Chinese collection (GDGM 47122) shares a remarkable resemblance with the African material of *P. losulus* described by Justo & al. (2011a), which differed by its larger basidiomata (30–90 mm broad), its longer thicker stipe (35–100 × 3–10 mm), and its partial brown pigmentation in the pleurocystidia; these differences could be due to environmental variation.

The ITS sequence of the African *P. losulus* (Goosens-Fontana 5273) is 99% identical (difference only two nucleotides) to those of the Chinese collection, suggesting that they are conspecific and that the species occurs in two continents. Some other species, e.g., *P. cervinus*, *P. longistriatus* (Peck) Peck, *P. romellii* (Britzelm.) Sacc., *P. velutinus* C.K. Pradeep & al., also have wide geographic distribution (Justo & al. 2011a, Pradeep & al. 2012, Menolli & al. 2015a). However, what dispersal mechanisms (either natural or anthropogenic)

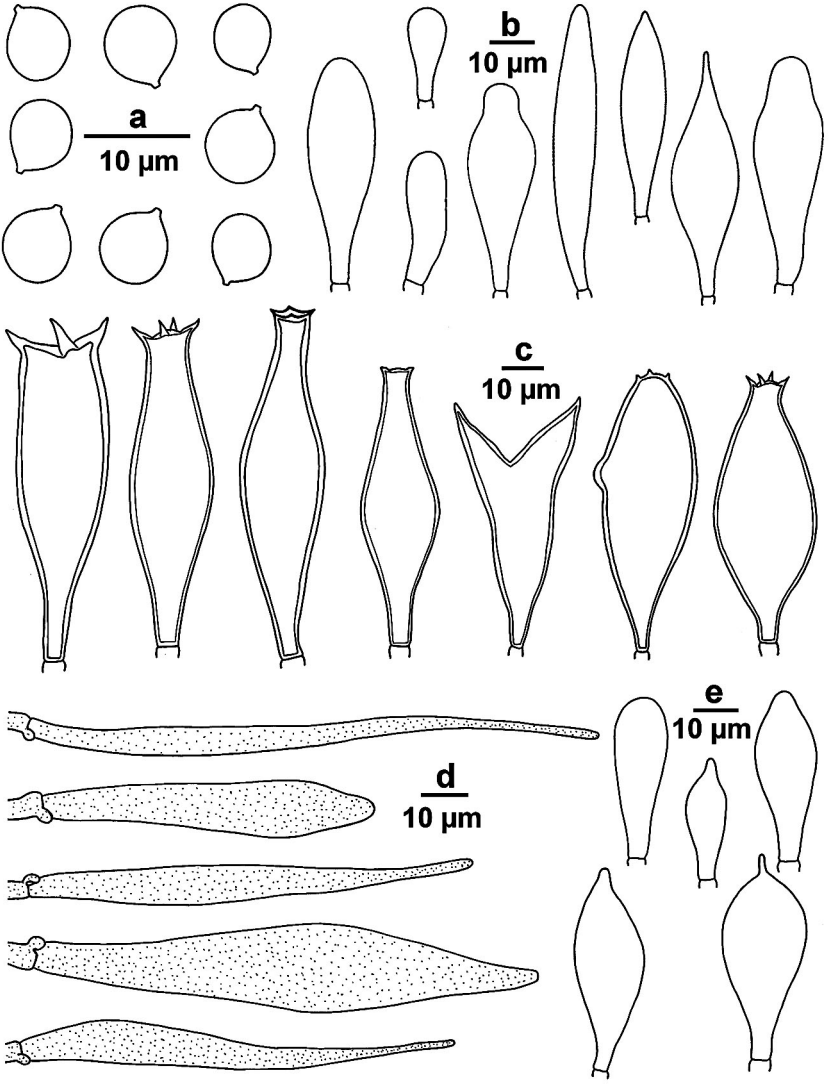

Fig. 3. *Pluteus losulus* (GDGM 47122). a. Basidiospores; b. Cheilocystidia; c. Pleurocystidia; d. Pileipellis elements (terminal cells); e. Caulocystidia.

are responsible for the distribution patterns of these species is still unknown. Gross morphology and molecular data support the identification of the Chinese collection as *P. losulus*.

The presence of clamp connections in *P. losulus* is not surprising, as clamps are found in several species of *P.* sect. *Pluteus*. However, the combination of such features as the hooked and partially pigmented metuloidal pleurocystidia (partial pigment absent from the Chinese specimen), variably sized and shaped cheilocystidia, frequent presence of caulocystidia, and the presence of clamp connections make it distinct within *P.* sect. *Pluteus* (Justo & al. 2011a). *Pluteus losulus* can be compared morphologically with some sub-basal (*P. atromarginatus* (Konrad) Kühner and *P. atrofibrillosus* Vellinga & Justo) and basal (*P. spinulosus* Murrill) taxa. However, *P. atromarginatus* is distinctive in *P.* sect. *Pluteus* because of its relatively large basidiomata (25–100 mm) with fibrillose-squamose pileus, dark brown lamellar edge, very thick-walled (5 μm) pleurocystidia, and association with coniferous trees (Justo & al. 2014); *P. atrofibrillosus* has a densely fibrillose/squamulose pileus, relatively shorter and narrower basidiospores (5.5–7.5 × 4.0–5.5 μm; mean 6.8 × 5.1 μm), and pleurocystidia predominantly with 2 apical hooks (Justo & al. 2014); and *P. spinulosus* is distinguished by its brown basidiomata, usually without apically hooked pleurocystidia, and absence of caulocystidia (Banerjee & Sundberg 1993; Xu & al. 2015).

The molecular study of *Pluteus* by Justo & al. (2011a) showed *P. losulus* together with *P. atromarginatus* as a basal clade in *P.* sect. *Pluteus*. However, in the infrageneric taxonomy of *P.* sect. *Pluteus*, *P. losulus* was separated from *P. atromarginatus* clade, forming an independent clade in which *P. losulus* is placed as the earliest diverging member for this section (Justo & al. 2011b). With the inclusion of ITS sequences of *P. spinulosus* from Bolivia (GenBank KJ009777) and India (GenBank KY630516) that often appear in the phylogeny as a basal clade to *P.* sect. *Pluteus* with moderate BS support, where *P. losulus* has an ambiguous position (preliminary analyses, data not shown) that might be due to lack of significant branch support. In this study, however, *P. losulus* recovered as a basal clade, while *P. atromarginatus* and *P. spinulosus* are poorly supported as close sister clades (Fig. 1). Therefore, the basal clade/taxon of *P.* sect. *Pluteus* remains unclear because neither the *P. spinulosus* clade nor the *P. losulus*/*P. atromarginatus* clade is strongly supported (Fig. 1). In order to establish the ancestral taxon/clade of *P.* sect. *Pluteus* with certainty, more taxa should be sampled from diverse areas.

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